***Alexandrium fundyense* resting cysts in the Gulf of Maine and the Bay of Fundy**

Final Report
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Introduction. *Alexandrium fundyense,* commonly referred to locally as the New England "red tide" organism, is a harmful algal bloom species that requires careful management of shellfish resources to prevent Paralytic Shellfish Poisoning (PSP) in New England and Canadian coastal waters. A multi-investigator, multi-disciplinary, NOAA-funded research program (GOMTOX) is studying the dynamics of toxic *Alexandrium fundyense* blooms in the Gulf of Maine (GOM) and the Bay of Fundy (BOF). One continuous element of that effort has been the collection of *Alexandrium fundyense* resting cysts that are deposited to the sediments following each bloom and serve as an inoculum for subsequent blooms each spring. To date, seven 11-day cyst cruises have been completed each autumn since 2004. Funding for this effort has not been continuous however, as various research projects have supported the work. In 2009 and 2010, the annual cyst cruises and the analysis of samples and data were funded through a partnership between NOAA and the affected New England coastal states.

Background. Over the past several years, an important management tool for red tides or toxic *Alexandrium* blooms in the Gulf of Maine region has been a numerical model of bloom dynamics developed at the Woods Hole Oceanographic Institution (WHOI). Each week during the bloom season, this model is run, providing a "hindcast" simulation of *Alexandrium* distribution and abundance for the preceding weeks and months, as well as a one-week short term forecast based on weather predictions. The model is initiated from a map of *Alexandrium* cyst abundance in Gulf of Maine bottom sediments and a new map is produced each year from the samples collected from annual cyst surveys.

Cysts are an important part of the organism's life cycle. As part of the life cycle, cysts are form when asexually dividing vegetative cells in the overlying bloom waters undergo sexual reproduction and produce new cysts that are deposited to the surface sediments. Those cysts form "seedbeds" that germinate and inoculate the overlying waters in the following year(s) to re-establish subsequent blooms. Past studies have shown that the abundance of cysts in the region varies dramatically year-to-year, and that the cyst abundance measured during the fall or winter in one year is a first-order predictor of the regional bloom magnitude in the next year. This knowledge, as well as the observation of a very high cyst abundance in 2007, led to a seasonal forecast of a major red tide in the Gulf of Maine in 2008, a forecast that was borne out with extensive shellfish closures in Maine, New Hampshire, and Massachusetts, as well as offshore federal waters.

Objectives. The primary objective of this work was the collection of surface sediments for the enumeration of benthic cysts of the toxic dinoflagellate, *Alexandrium fundyense*, in the GOM and the BOF. The new cyst data will add to our documentation of the inter-annual and inter-decadal changes in the overall cyst abundance and distribution pattern compared to earlier survey results. More importantly from a management perspective, a critical part of the GOMTOX modeling effort is using the fall 2009 and 2010 cyst data as the source input to provide a seasonal "outlook" of the potential for the bloom intensity in 2010 and 2011. Short-term bloom "nowcasts" are also currently provided each week assimilating near-real time hydrographic and atmospheric data into the models as noted above. The weekly forecasts (still considered a research product) are available to shellfish managers via the Northeast PSP listserve. To produce the seasonal bloom outlook and the weekly nowcasts during the bloom season, new cyst data is needed each fall from a cruise that samples the cyst population after the cysts have settled to allow sufficient time for those samples to be examined and the model analysis to be implemented.

A secondary objective of the survey was to determine if *A. fundyense* cells were present in the region during the fall period by examining surface water samples at each cyst coring station. For example, during the 2007 cyst survey, up to 20-30,000 cells/L were recorded in the Bay of Fundy and offshore of the southwestern Nova Scotia coastline, indicating that an intense and continuing summer/fall bloom along the Maine and Canadian coastline may have been responsible for the high cyst yield found in the western GOM sediments that year.

A final objective of the project was to statistically analyze all past cyst maps (1997, 2004 – 2010) to identify common features, and to use this analysis to optimize (minimize) the sampling regime for future surveys.

Methods. The R/V *Oceanus* cruise (OC458) sampled sediments from > 100 stations in the region over an 11-day period (October 15-25, 2009). The R/V *Endeavor* cruise (EN486) sampled sediments from > 100 stations in the region over an 11-day period (October 13-23, 2010). At each cyst station, undisturbed surface sediments were collected using a Craib Corer. All cores were processed on board into two layers (top 1cm and the underlying 1-3cm layer). Each sample layer was sonified and sieved to collect the particles in the <100µm and >20µm size fraction. The processed sample was preserved using formalin (5% final), then exchanged into methanol for storage until later microscopic analysis. The cyst samples were enumerated using a primulin-staining technique routinely performed at the WHOI lab in Woods Hole, MA. After several months of microscopic analysis, the cyst data from both layers were analyzed for each year and provided for the modeling effort to derive the seasonal outlooks.

For the statistical analysis of past cyst maps, the raw cyst data were given to statisticians in the WHOI Marine Policy Center. They had examined the data from several perspectives. The current approach is to test the hypothesis that there is a generic or "baseline" cyst distribution in which the cyst abundance at each station is fixed relative to other stations. In this approach, this baseline distribution can then be scaled upwards or downwards by a fixed factor based on sampling at a number of key stations. Thus the relative distribution of cysts is the same from year to year, but the magnitude of the total cyst abundance varies.

Results

Cyst surveys. Figure 1 shows the abundance and distribution of the cyst population in the fall of 2009 and 2010. The overall general distribution is not unlike previous surveys, but there are critically important differences in the two years. In 2009, cyst concentrations (0-1cm) in the region were the highest ever measured with total abundance more than 60 percent higher than what was observed prior to the historic red tide of 2005. At several stations, the concentration exceeded 5000 cysts cm^{-3}. Furthermore, the abundance in the Bay of Fundy was about 2x greater than previous observations. The cyst bed also appears to have expanded to the south evidenced by a continuous population of benthic cysts (>1000 cysts cm^{-3}) that extended from Penobscot Bay to the offshore waters east of Cape Ann, Massachusetts and Stellwagen Bank. While it is not unusual to observe >1000 cysts cm^{-3} along the mid-Maine coast, it is anomalous to observe >1000 cysts cm^{-3} offshore of New Hampshire and as far south as Cape Ann and Stellwagen Bank.

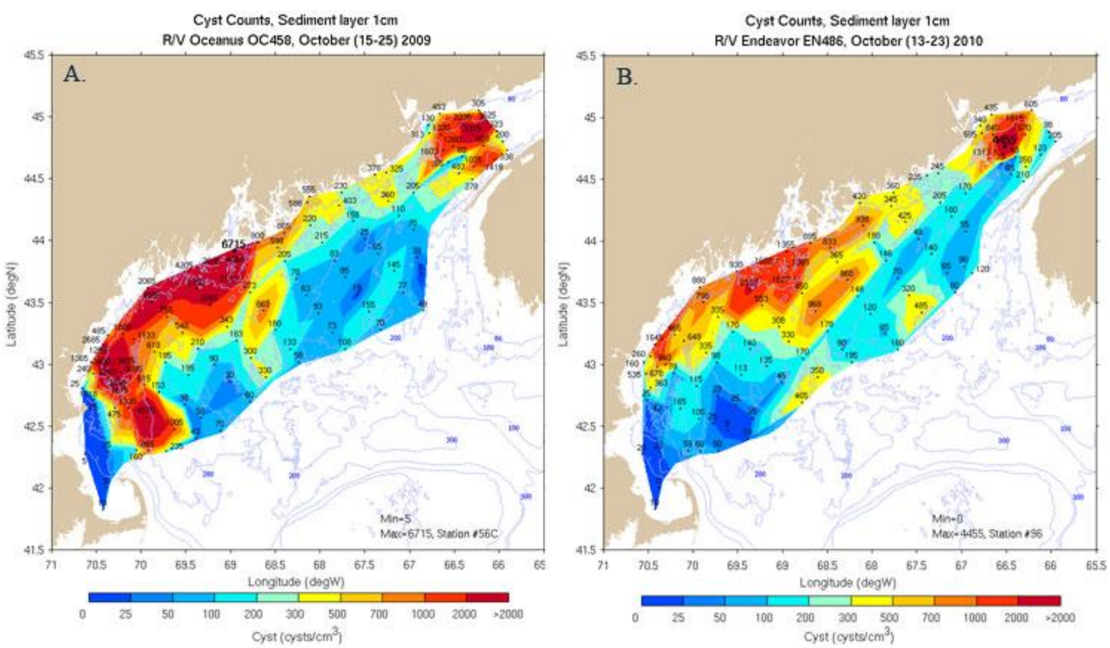

Figure 1. Cyst abundance in the Gulf of Maine (0-1 cm) – a) 2009; b) 2010.

In 2010, the overall general distribution shows a modest amount of cysts present in coastal sediments, not as high as the abundances prior to the major red tides of 2005 and 2008. Comparing this map with the one for 2009, we note that the southern extension of the population (offshore waters of Cape Ann, Massachusetts and Stellwagen Bank) for the most part, disappeared in 2010.

Statistical analysis of cyst maps. Figure 2 shows three years of cyst maps from direct microscopic counts, and three years of "simulated" cyst maps, in which the derived baseline cyst distribution was scaled upwards or downwards based on a factor determined by averaging the departure from the baseline counts at each station from the actual counts for that year. We are very pleased at the general correspondence between the maps for each year, generated in two very different ways. A comparable analysis has been done for 9 years of cyst maps, and results are similar.

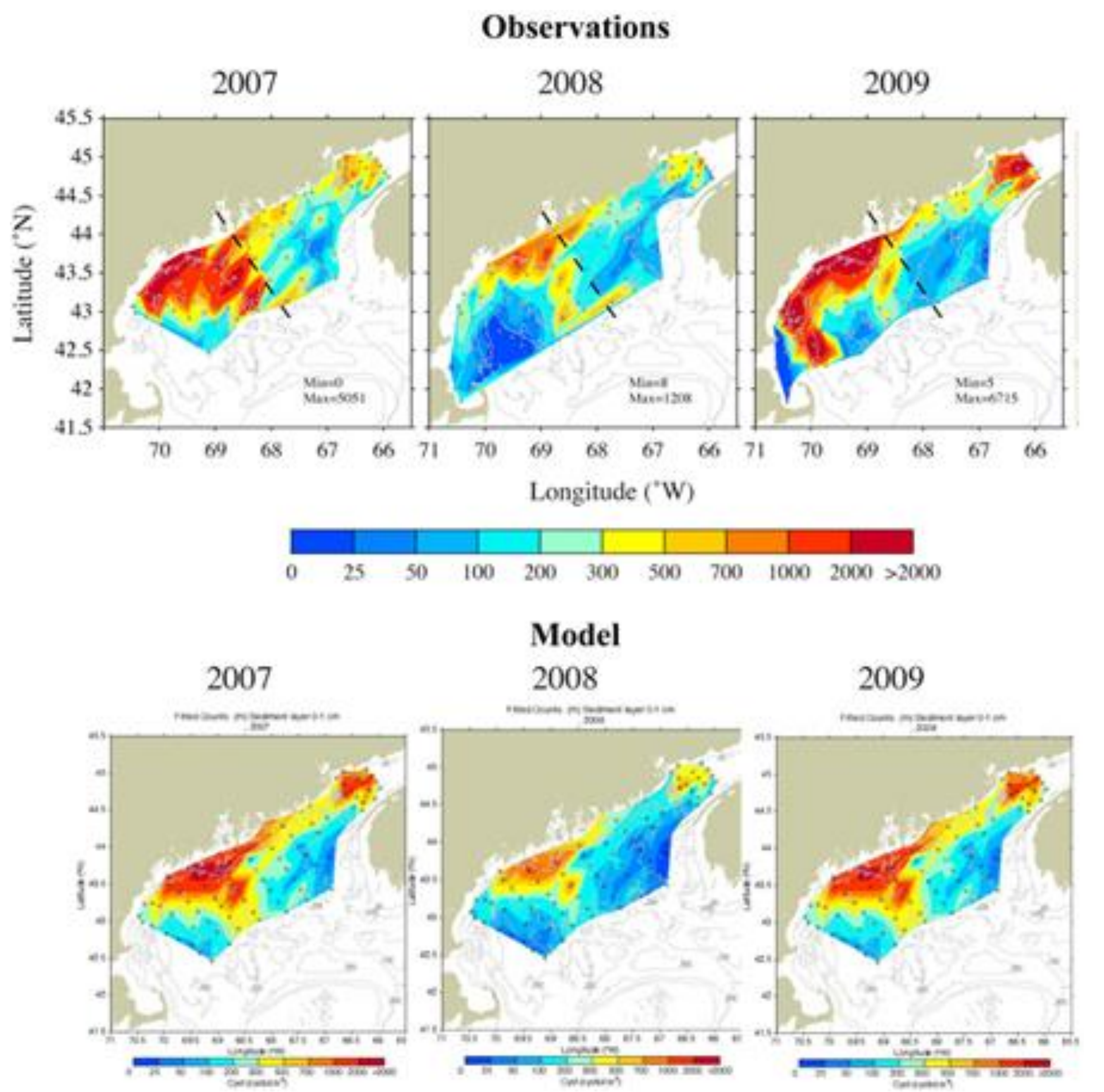

Figure 2.

In a follow-on to the statistical analysis of the system maps that we hope will allow us to have much smaller and more efficient system surveys in the future, the statistically-derived maps have been used in a modeling effort to see if the simulations that result are similar to those obtained with a full system map. Our team has developed a statistical model for cyst abundance based on the 78 stations that are common to all the surveys. The statistical model appears to capture a large fraction of the observed interannual variability (Figure 3A). Of course the statistical model provides only an approximation of the observations, and as such there are overpredictions in some years and underpredictions in others (Figure 3B,C). Moreover, the subset of 78 stations used to construct the model is not a perfect proxy for the total number of cysts in the domain, and this can mask some of the interannual variability. For example, the 78-station subset overestimates the total in 2007, whereas it underestimates in 2009 when there are more cysts farther south in the domain than previously observed.

In order to assess the utility of the statistical model for driving the 3D coupled physical-biological forecast model for *A. fundyense*, we compared hindcasts for the years 2005-2010 based on cyst maps using all the observations (Figure 3A, top row) to hindcasts using cyst maps generated by the statistical model (Figure 3A, bottom row). The results of the hindcasts are quite similar in terms of the Gulf-wide time-averaged cell concentration (Figure 4). Interestingly, the biggest discrepancies occur in the years in which the 78-station approximation to the total abundance has its largest errors (fall 2007 and fall 2009.

The modeled cyst distribution for each of the study years was used as input to our *Alexandrium* population dynamics model and results compared to those obtained using the full cyst map for that year. Comparisons were very good (Figure 4), giving us confidence that the statistical approach has good promise for reducing the sampling schedule. To this end, the project team has met to plan the manner in which the approach can be used to simplify sampling. The statisticians are examining the different subsets for the 78 stations to determine the error introduced by subsampling. We plan to specify an acceptable level of error (e.g., 20%) and this will then determine the number of stations that are to be sampled. Even though statistically, it does not matter which stations are used to satisfy this target criterion, there are other reasons to choose certain areas or stations. For example, cyst abundances are much higher off of Casco Bay, and thus counting statistics are much better than is the case for areas with much lower cyst abundances. These areas might also be easily reached with small vessel cruises leaving from Portland, ME, for example. The same can be said for the Bay of Fundy, as it is another high density area.

These initial results are encouraging, insofar as future forecasts could be made using a reduced set of cyst observations together with the statistical model for estimating the regional distribution. These activities are particularly timely, in that severe budget cuts to NOAA HAB programs, including the ship time budget, may require that the cyst mapping survey planned for fall, 2012, be reduced in scale. In that instance, we would be directly utilizing the reduced patterns of stations and transects derived from this project.

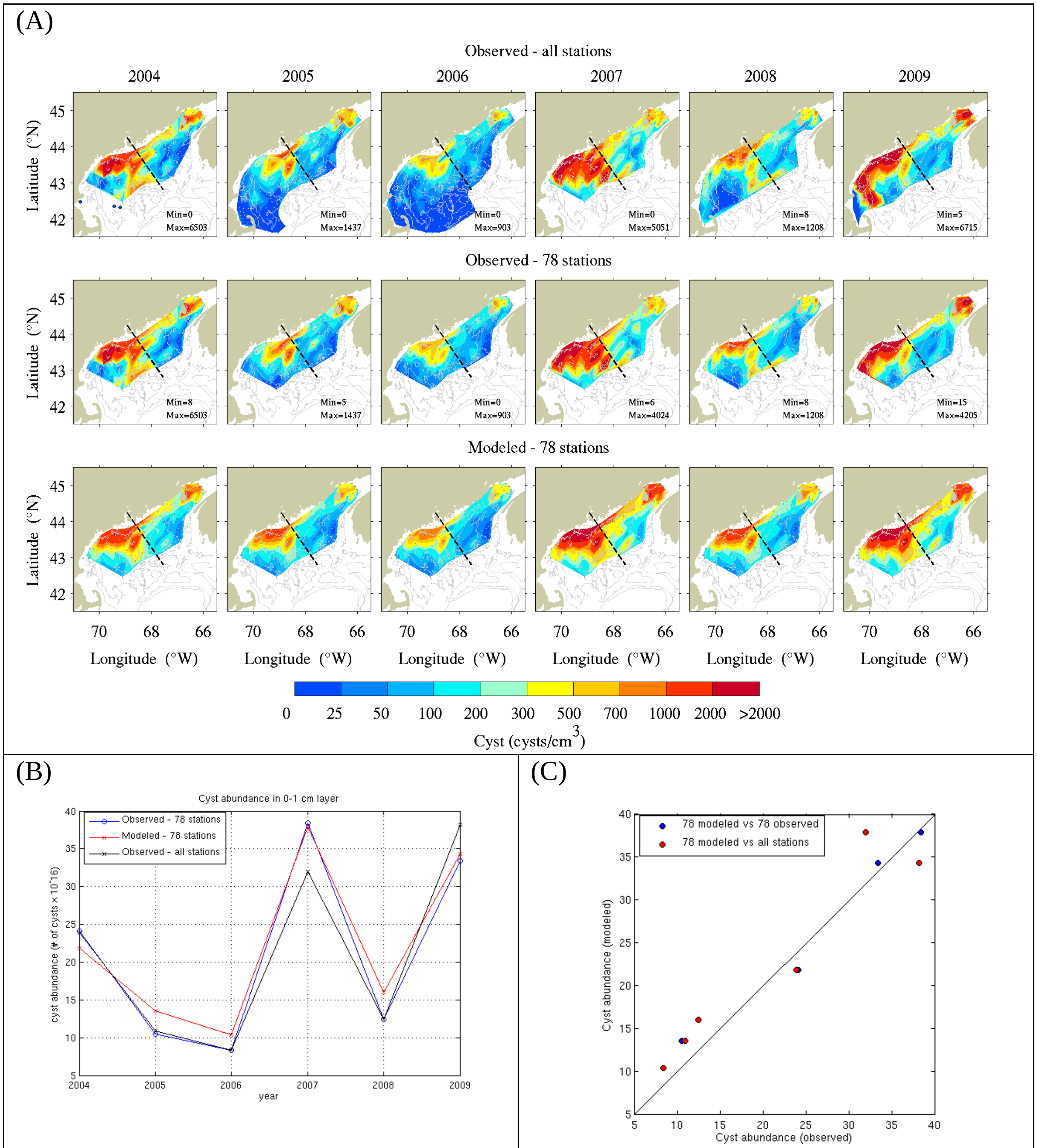

Figure 3. (A) Time-series of cyst maps using all available stations (top), the 78 stations that all years have in common (middle), and the simulated distribution of cysts based on Solow and Beet's statistical model based on those 78 stations (bottom). (B) Time-series of observed and predicted cyst abundance 2004-2009. (C) Scatterplot of simulated and observed cyst abundances, 2004-2009.

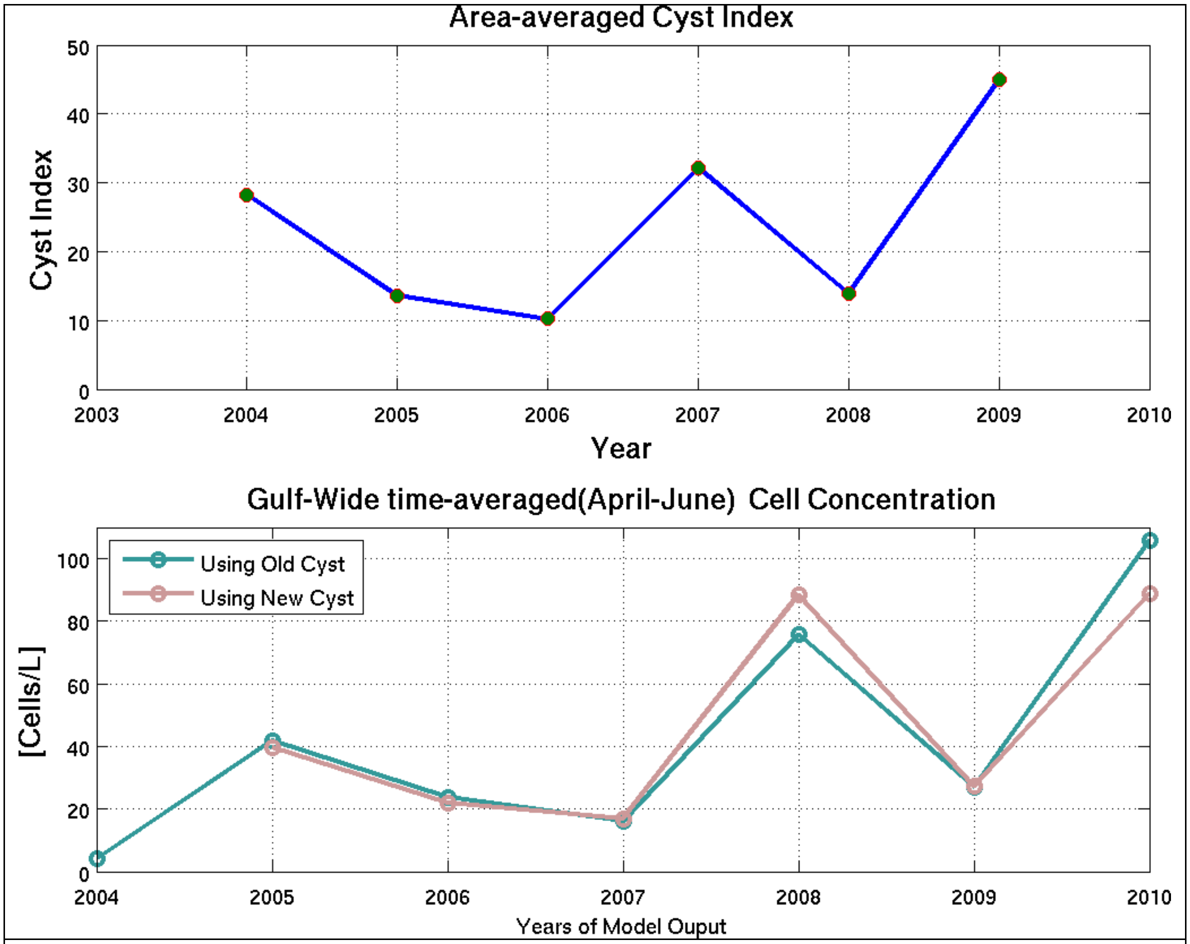

Figure 4. Time series of cyst abundance (top) and Gulf-wide time-averaged (April-June) cell concentration (bottom) predicted from the 3D physical-biological model used to make forecasts in the region. In the lower panel, “old cyst” refers to the cyst maps derived from the entire data set (Figure 1A, top row); “new cyst” refers to the statistical model of cyst distributions based on the subset of 78 stations (Figure 1A, bottom row).

Summary

The large-scale system maps obtained during this project (Figure 1), as well as preceding projects are being analyzed, and the data corporative into two scientific publications examining cyst dynamics during *Alexandrium* blooms and the new methods for cyst mapping based on our statistical analysis. This work was also highlighted in a talk at the *Sixth Symposium on Harmful Algae in the U.S.*, held in Texas last November.

The two regional system maps obtained during this project are of great importance in our study. This cyst time series documents two major cyst deposits in the system: one in the Bay of Fundy and the other offshore of mid-coast Maine (Figure 3A, top row). Whereas cyst abundance is relatively stable over time in the Bay of Fundy, the mid-coast Maine cyst bed fluctuates by more than an order of magnitude. Cyst abundance in the western Gulf of Maine deposit is a surprisingly good predictor of the extent of downstream toxicity closures for 2005-2009, although in 2010 a water mass anomaly appears to have suppressed what would have otherwise been a severe bloom. It is also of note that an extension of the mid-coast cyst bed was documented in 2009 following a visible red tide n the Portsmouth area. Our initial fear was that this extension would lead to larger and earlier toxic blooms in NH and Massachusetts waters, due to the enlarged cyst seedbed. The 2010 mapping effort, however, demonstrated that the extension had disappeared, and the overall cyst distribution had resumed its “typical” shape.

At the present time, the statisticians are deriving sampling maps for different levels of accuracy and these will be soon be evaluated by the project team. A cyst cruise (funded by NOAA’s PCMHAB program) is scheduled for this fall. It is unclear at this time whether a full map will be obtained or an optimized map. This will depend on budgetary issues. Given the likelihood that there will be significant budget cuts, we may well use the reduced sampling strategy of necessity. This project has thus played an extraordinarily important role in helping us to sustain these important cyst surveying efforts, and in designing an optimized and thus less expensive sampling strategy as we move toward operational deployment of the Gulf of Maine HAB forecasting system.